

Annealing:

	Kinased Oligo 2680	Kinased oligo 2723	Control
Ann. Buffer	1 μ l	1 μ l	1 μ l
U-SS DNA (gene cloned)	1.5 μ l	1.5 μ l	1.5 μ l
H ₂ O	6.5 μ l	6.5 μ l	7.5 μ l

Anneal at 70°C for 2 min \rightarrow let cool to \sim 35°C over 30-40 min.

Synthesis:

Add to tubes.

1 μ l 10x Syn. Buffer
0.3 μ l T7 DNA Pol (USB)
1.0 μ l T4 ligase (LTI)

S' on ice, S' at room temp — 45 min at 37°C.

Add 90 μ l TE (10mM Tris pH8.0, 10mM EDTA).

Transfect DH5 α F' IR.

Transform DH5 α F' IR 100 μ l with 1.5 μ l ligation/synthesis mix.

30 min on ice

42°C for 40 sec.

\rightarrow Split 10% and 90% for 2680 and 2723
Keep 100% for the control.

Add 6 μ l lawn cells + 3 ml soft agar

- let the agar solidify

\rightarrow Incubate plates at 37°C.

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Issued & Understood by me,

Date.

Invented by

Date

Recorded by